JHR 38: 37–43 (2014)
doi: 10.3897/JHR.38.6562
www.pensoft.net/journals/jhr

RESEARCH ARTICLE

JOURNAL OF Hymenoptera RESEARCH
A peer-reviewed open-access journal
The International Society of Hymenopterists

The prevalence of the parasitic nematode *Sphaerularia* sp. in the overwintering gynes of *Parapolybia* spp. (Hymenoptera, Polistinae)

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Academic editor: *Jack Neff* | Received 4 November 2013 | Accepted 25 April 2014 | Published 12 June 2014

http://zoobank.org/AF74A7A9-12F5-418E-A239-C07280A474F5

Citation: Saito-Morooka F (2014) The prevalence of the parasitic nematode *Sphaerularia* sp. in the overwintering gynes of *Parapolybia* spp. (Hymenoptera, Polistinae). Journal of Hymenoptera Research 38: 37–43. doi: 10.3897/JHR.38.6562

Abstract

I report for the first time the parasitization of overwintering gynes of *Parapolybia* species by the entomogenous nematode *Sphaerularia* sp. (Tylenchidae). The nematode was found in 42% of the overwintering wasp clusters examined but occurred in only 6% of individual wasps. The prevalence of parasitic nematodes among group hibernating hymenoptera is briefly discussed and compared with that in solitary hibernating hymenopterans.

Keywords

Parapolybia, group hibernation, gyne, parasitic nematode, Polistinae

Introduction

The parasitic nematodes of social hymenopterans include Mermithidae, *Pheromermis pachysoma* (von Linstow), for *Vespula* spp. (Poinar et al. 1975; Edwards 1980) and Tylenchidae, *Sphaerularia bombi* Dufour, 1837, for *Bombus* spp. (Poinar and van der Laan 1972; Poinar and Hess 1972; McCorquodale et al. 1998; Schmid-Hempel 1998) and *S. vespae* Kanzaki et al., 2007, for *Vespa simillima* Smith (Sayama et al. 2007). The genus *Sphaerularia* is characterized by the mature female having a large everted uterine sac as its reproductive organ. This is well developed in the host's metasoma (Poinar and

Hess 1972; Poinar and van der Laan 1972). Female nematodes have a negative effect on their host's fitness, such as the sterilization of queens in *Vespa simillima* (Sayama et al. 2007) and *Bombus* spp. (Poinar and van der Laan 1972; Pouvreau 1974) and the reduction of colony productivity in *Bombus hypnorum* (Linnaeus) (Röseler 2002). Following emergence, parasitized overwintered gynes (i.e., potential queens) of hornets and bees usually live a solitary life without founding a colony (Schmid-Hempel 1998; Sayama et al. 2007).

During their overwintering diapause, gynes of bees and hornets are prone to nematode infection by the adult infective form (= inseminated females). By the following spring, the uterine sacs of parasitic nematode females are developed. Instead of founding a colony, parasitized overwintered gynes look for potential hibernation sites and parasitic juveniles are released from the host's metasoma into hibernacula by late summer (Poinar and van der Laan 1972; Schmid-Hempel 1998; Sayama et al. 2007, 2013).

In temperate regions, social hymenopterans have two principal modes of hibernation (but *Apis* spp. do not hibernate): independently or in groups. In *Bombus*, *Vespa*, and some *Polistes* species a single gyne, or few gynes, tunnel under tree bark, soil, or rotten wood before winter and diapause until the following spring (Schmid-Hempel 1998; Matsuura 1980). This mode is known as solitary hibernation. *Sphaerularia* nematodes have previously been reported only in solitary hibernating species. Eight species of *Polistes* and two species of *Parapolybia* occur in Japan. Three species of *Polistes* (*Po. japonicas* de Saussure, *Po. jokahamae* Radoszkowski and *Po. rothneyi* Cameron) and two *Parapolybia* species [*P. indica* (de Saussure) and *P. varia* (Fabricius)] are known as group hibernating species. In group hibernating species usually more than a dozen gynes and sometimes hundreds, overwinter together. *Polistes* group hibernating species sometimes use abandoned nests of vespid wasps (Gibo 1980; Matsuura 1980; Kojima 1993), while *Parapolybia* species usually use the hollow of a broadleaf tree as a hibernaculum (Matsuura 1980; Sugiura et al. 1983).

This is the first report of the infection of gynes of the group hibernating polistine wasp *Parapolybia indica* by parasitic nematodes. In this study, I describe the prevalence and condition of wasps infected by parasitic nematodes in their overwintering clusters and briefly compare them with solitary hibernating hymenopterans.

Materials and methods

Sample collection of overwintering individuals

Parapolybia wasps were collected in Japan from October 2009 to April 2010 in the forest park of Mito city, Mito (36°25'N; 140°22'E, alt. 130 m, site A), Ibaraki Botanical Garden, Naka (36°29'N; 140°26'E, alt. 60 m, site B), Hitachi Ohmiya (36°39'N; 140°16'E, alt. 160 m, site C), the Seminar House of Ibaraki University, Daigo (36°49'N; 140°23'E, alt. 190 m, site D), Ibaraki Prefecture; in October 2012 in Rissho University, Kumagaya (36°06'N; 139°22'E, alt. 50 m, site E), and Nourin park,

Fukaya (36°06'N; 139°17'E, alt. 90 m, site F), Saitama Prefecture. Wasps were collected in the following four phases: phase I) the pre-hibernating phase, which occurs shortly after mating and colony break up, in which females temporarily gather at a nest (possibly at their natal nest) or on plant leaves from late August to mid-October (sites D, E, and F); phase II) the early hibernating phase, in which females alternate between hibernacula from late October to mid-November (sites A and D); phase III) the late hibernating phase, in which females remain in the hibernaculum from mid-January to early February (sites A, B, and C); and phase IV) the precolony founding phase, which occurs shortly after emergence of gynes from hibernation in April (sites A and B).

Most overwintering wasps were found in or near hollows of broadleaf trees such as *Quercus serrata* Murray, *Q. acutissima* Carruthers, or *Prunus jamasakura* Siebold, except for cluster no. A-1, which was occurred under wooden stairs in the park. Wasps resting around the entrances of the holes were collected with forceps during the phase II to IV at sites A and B. For wasps deep inside a tree hollow, the tree was cut off or the entrance of the hollow was bored out, and wasps were extracted with long forceps and/or a wire during phase II at site D and during phase III at sites A and B. The collected wasps were transported in a cool box with ice and stored in that box until dissection later the same day.

Dissection of wasps

The metasomas were removed from live wasps, or wasps killed by 60% ethanol, and dissection was performed on a glass Petri dish under a binocular microscope. The metasomas were examined for the presence of nematodes. Parasitic nematodes were categorized into the following three stages: juveniles (small individuals with of indeterminate sex); uteria (mature adult females with developed uterine sacs); and infective forms (adult females without such sacs) (Fig. 1). Voucher specimens of nematodes and wasps were deposited in the Natural History Collection of Ibaraki University.

In addition, wasps were examined for the presence of fat bodies, sperm in the spermatheca, and the degree of wing wear. Wasps with fat bodies, sperm in the spermatheca, and a lack of wing wear were classified as gynes, whereas individuals lacking fat bodies or sperm in the spermatheca, and with worn wings were classified as workers.

Results

Overwintering *Parapolybia*

For *Parapolybia indica*, three pre-hibernating clusters (gathered at the natal nest) in groups of 14, 16 and 99 (129 total individuals) were collected. And an additional 551 wasps were collected in phases II-IV from 23 additional hibernacula in the phase II–IV (Table 1). Solitary hibernating wasps were found at sites A, B, C, and D (n = 5). The

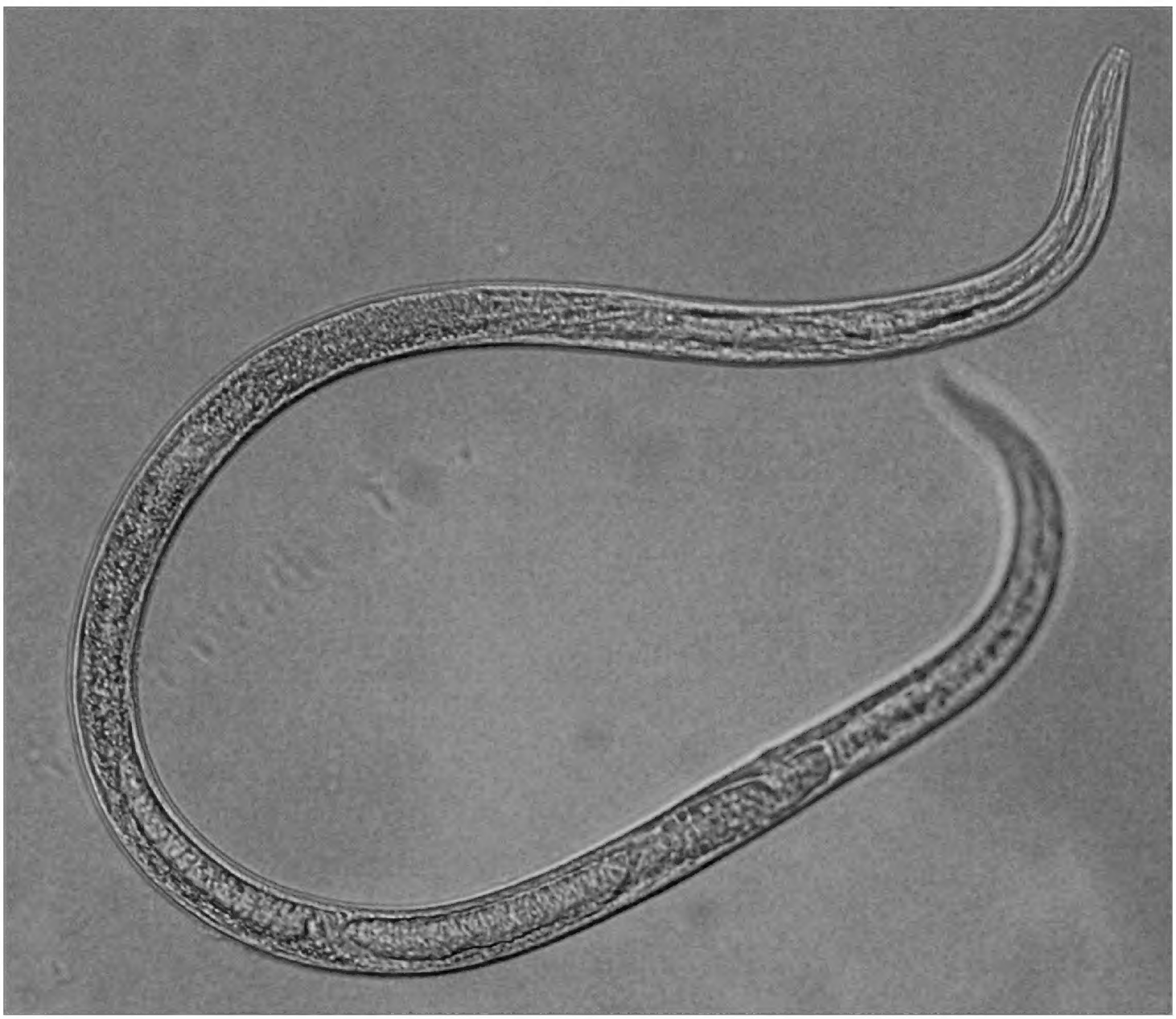

Figure 1. Photomicrograph of a parasitic nematode found in *Parapolybia*. It is considered a free-living or infective form of nematode because it lacks a well-developed everted uterus sac.

Table 1. The number of collected overwintering clusters and wasps. The number of clusters/wasps infected with *Sphaerularia* sp. is shown in parentheses. The breakdowns of the number of infected wasp individuals in each overwintering phase are shown in four columns on the right side.

Species	Site	Location	No. of clusters	No. of wasps	Overwintering phase			
					Phase I	Phase II	Phase III	Phase IV
P. indica	A	36°25'N, 140°22'E, alt. 130 m	17 (8)	408 (28)	-	231 (14)	138 (11)	40 (3)
	B	36°29'N, 140°26'E, alt. 60 m	2 (1)	63 (7)	-	-	55 (5)	8 (2)
	C	36°39'N, 140°16'E, alt. 160 m	1 (0)	1 (0)	-	-	1 (0)	-
	D	36°49'N, 140°23'E, alt. 190 m	4 (2)	93 (7)	14 (0)	79 (7)	-	-
	E	36°06'N, 139°22'E, alt. 50 m	1 (0)	99 (0)	99 (0)	-	-	-
	F	36°06'N, 139°17'E, alt. 90 m	1 (0)	16 (0)	16 (0)	-	-	-
	total		26 (11)	680 (42)	129 (0)	310 (21)	193 (16)	48 (5)
	Percentage		42.3%	6.2%	0.0%	6.8%	8.2%	10.4%
P. varia	D	36°49'N, 140°23'E, alt. 190 m	1 (0)	102 (0)				

number of individuals found in a hibernating cluster varied from 2 to 192 (n = 18; mean ± SD = 30.44 ± 44.98, median = 11).

After dissection, thirteen individuals of *Parapolybia indica* were classified as workers (site A, n = 4; site D, n = 1; site E, n = 7; site F, n = 1). They were collected in phases I-II and had no parasitic nematodes in their metasomas. Seven wasps collected before the phase III had no fat bodies or sperm in the spermatheca, and their wings were slightly damaged (site A, n = 5; site D, n = 2). For such samples, the wings may have been damaged during collection, and/or they were recently emerged workers. The other *P. indica* (n = 660) and *P. varia* (n = 102) had fat bodies, sperm in the spermatheca, and a lack of wing wear and were classified as gynes.

Nematode-infected wasps and prevalence of parasitic nematodes

The total number of individuals infected by parasitic nematodes was 42. Such individuals were collected from their hibernacula during phases other than the phase I (Table 1). No phase I wasps were found to be nematode infected. All infected individuals were *Parapolybia indica* and were clearly classified as gynes. The prevalence (%) of parasitic nematodes in each overwintering cluster varied from 3.5 to 50.0 (median = 10.5). No statistical relationship was found between the cluster size and prevalence (correlation efficient r = -0.14, R ver.2.15.2, R Core Team 2012). The prevalence (% of infected clusters) increased through the season: for each phase, 0.0 in phase I, 6.8 in phase II, 8.2 in phase III, and 10.4 in phase IV. The overall percentage of nematode infected wasps was 6.2% (Table 1).

A single overwintering cluster of *Parapolybia varia* was found at site D. It was without nematode infection. This cluster was found in the same tunnel as a cluster of *P. indica*. However, each cluster was monospecific and no species mixing was observed in the tunnel. Thus, in this study, only individuals of *P. indica* were infected by nematodes.

Parasitic nematodes

Parasitic nematodes were found only in the hemocoel. The stages of nematodes found in the wasp metasomas varied based on the phases of the wasps at collection. Infected wasps collected from phase II had a large number of juveniles and one or two uteria in their abdomen. Uteria and infective forms (Fig. 1) were found in the metasomas of wasps from phase III clusters. Infected wasps from phase IV contained only uteria.

Discussion

For *Vespa simillima*, more than 60% of overwintered gynes were parasitized by *Sphaerularia vespae* (Sayama et al. 2007, 2013). Parasitized overwintered gynes do not found new colonies in the spring and early summer and probably are readily trapped by

baited traps (Sayama et al. 2007). Therefore, the overall percentage of parasitized females might have overestimated. For bees, the prevalence of *Sphaerularia bombi* varies among bumblebee species and the nesting season (McCorquodale et al. 1998). This study is the first report on the prevalence of parasitic nematodes in overwintering gynes of group a hibernating social hymenoptera. The percentage of parasitized females by *Sphaerularia* sp. in *Parapolybia indica* (6.2%) was lower than other known parasitic *Sphaerularia* species. The prevalence reported here is the percentage of parasitized gynes during overwintering season. Thus, it cannot be simply compared with that of parasitic nematodes in independently hibernating hosts reported previously. The possibility of parasitized wasps founding a new colony in the spring is still unknown.

Infection of hosts (bees and hornets) by parasitic nematodes of the genus *Sphaerularia* occur during the overwintering season in hibernacula such as soil and rotten wood (Poinar and van der Laan 1972; Kanzaki et al. 2007). All wasp individuals parasitized by nematodes are gynes. Infection by nematodes occurs during the period between adult emergence and emergence from hibernation. Thus, the first invasion of *Parapolybia* wasps by parasitic nematodes may also occur in the hibernaculum, as previously reported for solitary hibernating hosts. Juvenile nematodes hatch in the wasp's metasoma and are likely ejected from the host in the hibernaculum before the late hibernating phase (phase IV) of the host. In that case, this character is unique compared with other *Sphaerularia*. Elucidation of the detailed life cycle of parasitic nematodes and its impact on reproductive fitness of *Parapolybia* require further study.

Acknowledgement

The present study was supported by grants from the Japan Society for the Promotion of Science (no. 08J03623). I thank N. Kanzaki for the identification of parasitic nematodes.

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